The
Journal of Infectious Diseases

FOUNDED BY THE MEMORIAL INSTITUTE FOR INFECTIOUS DISEASES

VOL. 7 *January 15, 1910* NO. 1

A COMPARATIVE STUDY OF INTESTINAL STREPTOCOCCI FROM THE HORSE, THE COW, AND MAN.*

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INTRODUCTION.

THE first real clue to the systematic relationships of the streptococci was furnished by Gordon when he pointed out that these organisms could be separated into well defined groups by a study of their fermentative reactions in various carbohydrate media (Gordon, 1904; Gordon, 1905). Houston carried forward the application of Gordon's tests (Houston, 1905; Houston, 1906); and finally Andrewes and Horder (1906) founded upon all these results a rational classification of the genus into seven main types or species. The basis of their division was statistical or biometrical. It involved the examination of records of many individual cultures (1,200 in the case of the streptococci) and the study of the frequency with which various characters, or combinations of characters, occurred. Type centers, according to the statistical method, are defined by the occurrence of a large number of individuals with a given characteristic. These common types, among such variable organisms as the bacteria, may properly be considered as representing species, about which the rarer varieties are grouped.

* Received for publication October 22, 1909.

The chief characters of the species of streptococci thus defined by Andrewes and Horder are indicated concisely in the table below (Table 1). Strept. equinus is described as characteristic of the intestine of the herbivora. It was abundant in horse-dung and was the commonest form in the street air of London. All the other forms were primarily of human origin, Strept. mitis and Strept. salivarius from the normal throat, Strept. fecalis from the normal intestine, and Strept. pyogenes and Strept. anginosus from diseased conditions.

TABLE 1.

TABULAR CLASSIFICATION OF THE STREPTOCOCCI.
(Andrewes and Horder, 1906.)

SPECIES	CHARACTERISTICS								
	Milk Clot	Neutral Red	Saccharose	Lactose	Raffinose	Inulin	Salicin	Coniferin	Mannit
Strept. equinus	−	−	+	−	−	−	+	+	−
" mitis	−	−	+	+	−	−	+	−	−
" pyogenes	−	−	+	+	−	−	+	−	−
" salivarius	+	+	+	+	+	−	−	−	−
" anginosus	+	+	+	+	+	−	−	−	−
" fecalis	+	+	+	+	−	−	+	+	+
Pneumococcus	+	−	+	+	+	+	−	−	−

One of the most interesting practical points about this classification lies in the fact that all the six streptococci of human origin ferment lactose, while Strept. equinus, which Andrewes and Horder hold to be characteristic of the intestine of the herbivora, fails to do so. If a general distinction between human and animal streptococci could be demonstrated it would prove of much practical, as well as theoretical importance. Sanitarians are continually seeking, and seeking in vain, for some criterion by which sewage pollution of human origin may be distinguished from the surface wash of streets and agricultural land. The colon bacilli from the intestines of various warm blooded animals have been shown in many investigations—the latest and most exhaustive coming from Dr. Bettencourt's laboratory at Lisbon (Ferreira, Horta, and Paredes, 1908; Bettencourt and Borges, 1908*a* and 1908*b*)—to be identical. If the streptococci should prove to be characteristic it would greatly aid the water analyst.

The work of Gordon, and Andrewes and Horder, to which reference has been made, pointed to lactose as the best differential medium

for distinguishing human and animal streptococci. These investigators showed clearly that a large proportion of streptococci of human origin ferment lactose while most of those derived from horse-dung or street air fail to do so. On the other hand Houston (1906) in the examination of cow-dung obtained widely different results. Eighty-five per cent of his cultures from this source fermented lactose; but their reactions in mannit, raffinose, and neutral red were somewhat characteristic. Mannit was fermented by 24 per cent of the human cultures and by none of the bovine strains; raffinose was fermented by 32 per cent of the human and by 74 per cent of the bovine cultures; neutral red was reduced by 39 per cent of the human strains and by none of those from cow-dung.

These results suggest that both the horse and cow have characteristic types of intestinal streptococci which differ from each other and from those commonly found in the intestine of man. Our object in the present investigation was to confirm this conclusion by the study of a new series of cultures from the three sources. In particular we wished to obtain quantitative results with regard to the amount of acid formed in various media. This aspect of the question has been ignored by all the English observers, who are content merely to classify their cultures as fermenting or non-fermenting. Recent studies of the Coccaceae as a whole have shown however that exact quantitative data are of much value in classification (Winslow, 1908). We chose dextrose, lactose, raffinose, and mannit as the carbohydrates to be tested. Dextrose serves as a type of the simple sugars which are fermented to some extent by almost all streptococci. Lactose and raffinose represent, respectively, the aldehydic and anhydric groups of sugars. Mannit represents the alcohols. Three hundred and two strains of streptococci, from human, equine, and bovine feces, were grown in broths containing these carbohydrates and the resulting acidity in each case was determined by titration. The results as analyzed below indicate definite and characteristic differences between the streptococci from the three species of animals.

Methods.—All the streptococci studied were isolated from fresh feces, collected in a sterile tin can with tight-fitting cover. Human feces were deposited directly in the can while horse-dung and cow-dung were taken from the stable floor within a few minutes after ejection. Streptococci were isolated from 15 samples of human feces, 12 samples of horse-dung, and 22 samples of cow-dung. In all cases care was

taken to remove the outer layer of the feces and to take samples from the interior in order to avoid contaminations from stable floor or air.

The attempt was made at first to isolate the streptococci by inoculating a loop of feces into dextrose broth, incubating for various periods from 4 to 96 hours, and then plating on agar. This procedure is effectual in isolating streptococci from polluted water; but with feces it utterly failed in our hands. Spore-forming bacilli were found upon the plates or no growth appeared at all. It is probable that the streptococci were overgrown by other bacteria, perhaps by forms like B. acidophilus.

We therefore adopted the method of direct plating on agar without any preliminary enrichment whatever; and this proved generally successful. Two or three heavy platinum loopfuls of feces were well shaken up in 10 c.c. of sterile water and two or three loops of the resulting suspension carried over to a tube of melted agar. This was poured into a Petri dish and incubated at 37° for 24–48 hours. Agar streak cultures were made from minute round colonies on these plates and the appearance of these streaks after 24–48 hours at 37° was generally characteristic. Streaks showing a faint veil-like growth or a few dotted colonies or a thin line confined to the streak itself were almost always streptococci. In isolating the first 210 strains a microscopic examination was made. Smears were stained for 30 seconds with cold carbol fuchsin and cultures showing chains, pairs, or single cocci were accepted. In every one of these 210 cases the microscopic examination confirmed the conclusion based on the macroscopic appearance; and we are confident that the characteristic growth of the streptococci can be easily recognized by the experienced observer. Heavy growths on the streak always proved to be bacilli. Chromogenic, vigorously growing staphylococci were not found at all.

Streptococci were least easily detected on the plates made from cow-dung. The atter showed a great many small white colonies from which selection was difficult. Four out of five cultures streaked from cow-dung proved to be bacilli while not one out of five of the cultures isolated from horse-dung as streptococci failed to prove so.

A rough estimate was made of the total number of bacteria in one sample of each of the three kinds of feces, by plating high dilutions on agar, and incubating at 37° for 48 hours. The number of streptococci was estimated by isolating and identifying colonies which seemed characteristic. The sample of human feces showed an average of 8 million bacteria per gram of wet feces and 2,500,000 were streptococci. The proportion of streptococci is somewhat higher than that reported by MacNeal, Latzer, and Kerr (1909) who found that streptococci made up from 10 to 20 per cent of the total bacteria present. This is perhaps because MacNeal and his associates determined their total count by direct enumeration under the microscope, while our "total" is only the agar count at 37°. It is probable that most of the intestinal cocci grow on ordinary laboratory media, while we know that many bacilli and spirilla do not. In the sample of the horse feces we found an average of 3 million bacteria, of which nearly half were streptococci. This corresponds with our general experience of the high proportion of streptococci in equine feces. Cow-dung on the other hand showed in the sample examined an average of 100 million bacteria per gram of wet feces, of which 10 million were streptococci. This small ratio of streptococci (altho the actual numbers were high) explains the difficulty experienced throughout in isolating streptococci from cow-dung.

An interesting individual case was met with in the study of human feces, in which streptococci could rarely be found at all and in which all bacteria growing on ordinary

media were present in comparatively small numbers. Altogether the feces of 11 different persons were examined and streptococci were easily isolated in 10 cases. From this one individual, 7 samples were taken and 67 plates were made by the usual method (a loopful or two from feces to 10 c.c. water and a loopful or two of water to a plate). Only 22 out of the 67 plates showed growth and but 6 showed streptococci.

By the methods described above 302 cultures of streptococci were obtained in all, 116 from man, 100 from the horse, and 86 from the cow. As soon as a streak proved positive it was inoculated into broth tubes containing the four carbohydrate media tested. These were incubated for 72 hours at 37° and then titrated. Five c.c. of the liquid in each tube was measured out into a graduate, mixed with 45 c.c. of distilled water, and titrated in the cold against *n*/20 NaOH, using phenolphthalein as an indicator. Blank titrations were made at the same time on sterile tubes of the same batch and their results subtracted from those obtained for the inoculated tubes. The media used were in all cases made up with one per cent Witte's peptone, 0.25 per cent Liebig's beef extract, and one per cent of the carbohydrate to be tested.

Positive acid reactions were generally associated with obvious appearances of growth in the form of profuse white sediment along the bottom and sides of the tubes. In a very few cases, however, acid was produced in tubes which appeared clear to the eye.

In the case of negative tubes, with no rise in acidity and no turbidity or sediment the lack of development might conceivably be due to a failure of inoculation. Great care was taken however to transfer as much of the cultures as could be carried on a platinum loop. The fact that obvious growth almost always occurred in the dextrose broth tubes shows pretty clearly that inoculation was not at fault. The contents of 49 of the clear tubes of various media were examined by plating out on agar. Three tubes showed many colonies, 15 showed 1 to 6 colonies, and 31 showed none. It may reasonably be assumed that in such cases the streptococci introduced had simply failed to develop and gradually died out on account of the lack of suitable carbohydrate pabulum, upon which these organisms appear to be highly dependent.

RESULTS.

The results of the individual titrations are given in tabular form at the end of the paper. The first column indicates the culture examined, and the second, the number of the sample of feces from which it was derived. The figures for acidity represent the difference between the value obtained for each culture and the value of an inoculated control incubated under similar conditions. The results are probably significant within 0.2 per cent acidity.

A clearer idea of the meaning of these results may be gained from an inspection of Table 2 in which they are grouped together into acidity-classes, and from Charts 1, 2, 3, and 4, which have been plotted from the values in Table 2.

Dextrose is fermented more or less vigorously by practically all the streptococci from human and equine feces, but the streptococci

of man produce a markedly greater amount of acid than those from horse-dung. The mode for the human cultures lies between 3.6 and 4.0 per cent; that for the equine cultures between 1.6 and 2.0 per cent. The streptococci from cow-dung exhibit two distinct and clearly marked types, one forming about 2.0–2.5 per cent acidity, approaching the value of those from horse-dung, and the other group, of about the same numerical importance, forming no acid at all.

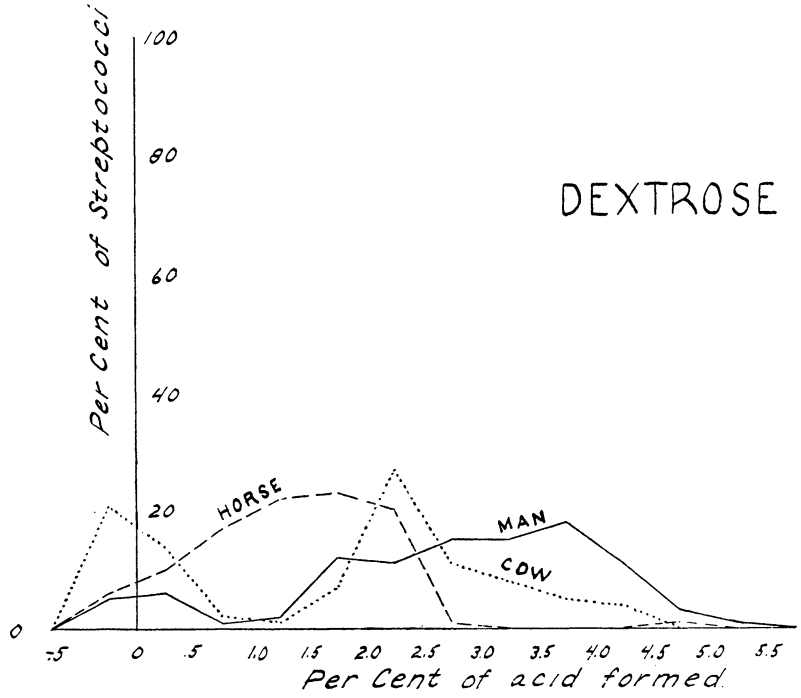

CHART 1.—Acid producing power of streptococci in dextrose broth.

In lactose the human and bovine cultures show two distinct types, one group yielding an acidity between 2.6 and 3.0 per cent, the other group producing no acid reaction at all. In the horse feces only the non-acid type is found.

Raffinose is not acted upon by any appreciable number of human or equine cultures. On the other hand, in the feces of the cow a small but clearly marked group appears, forming an acidity between 2.1 and 2.5 per cent.

Mannit is apparently not fermented by any important number of streptococci in the horse or cow but is acted upon by a small but definite group of the human strains.

TABLE 2.

STREPTOCOCCI GROUPED IN PER CENT ACIDITY CLASSES.

	−.5 0	.1 .5	.6 1.0	1.1 1.5	1.6 2.0	2.1 2.5	2.6 3.0	3.1 3.5	3.6 4.0	4.1 4.5	4.6 5.0	5.1 5.5
					Man (116)							
Dextrose	6	7	1	2	14	13	17	17	21	13	4	1
Lactose	20	24	2	5	17	11	24	12	1	0	0	0
Raffinose	34	75	2	1	0	1	2	1	0	0	0	0
Mannit	47	36	6	4	12	0	1	0	0	0	0	0
					Horse (100)							
Dextrose	6	10	17	22	23	20	1	0	0	0	1	0
Lactose	66	26	4	2	1	0	1	0	0	0	0	0
Raffinose	54	42	1	0	2	1	0	0	0	0	0	0
Mannit	62	36	0	1	1	0	0	0	0	0	0	0
					Cow (86)							
Dextrose	18	12	2	1	6	23	9	7	4	4	0	0
Lactose	27	14	1	0	8	8	20	8	0	0	0	0
Raffinose	36	26	0	3	6	11	3	1	0	0	0	0
Mannit	54	27	2	3	0	0	0	0	0	0	0	0

These results are compared in a general way with those obtained by Houston (1905, 1906) and Andrewes and Horder (1906) in Table 3. In classifying our own results for this table we have considered all results under 0.5 per cent as negative. The various investigations are

TABLE 3.

COMPARATIVE RESULTS OBTAINED BY VARIOUS OBSERVERS IN REGARD TO FERMENTATIVE POWER OF INTESTINAL STREPTOCOCCI FROM VARIOUS SOURCES.

SOURCE OF FECES	OBSERVER	NUMBER OF CULTURES	PERCENTAGE OF POSITIVE RESULTS			
			Dextrose	Lactose	Raffinose	Mannit
Human	Houston	300	..	76	32	24
Human	Winslow and Palmer	116	89	62	6	28
Equine	Andrewes and Horder	13	..	0	0	0
Equine	Winslow and Palmer	100	84	8	4	2
Bovine	Houston	100	..	85	74	0
Bovine	Winslow and Palmer	86	65	52	28	6

concordant with the exception that raffinose-fermenters in both human and bovine feces were less frequent in our observations than in those of Houston. Our results also indicate a somewhat lower percentage of lactose-fermenters. All the investigations show that streptococci from the human intestine generally attack lactose while some strains ferment mannit; bovine strains attack lactose or raffinose; and equine strains dextrose only.

In determining what types of streptococci are present in each species of animal, the correlation of fermentative power must be taken into account, as well as the activity of the organisms in each particular sugar. In the horse the problem is a simple one for all the organisms studied belong to a single type, characterized by a moderate acid production in dextrose broth and failure to attack either of the other carbohydrates. This is clearly the Strept. equinus described by Andrewes and Horder. In the human and bovine feces, on the other hand, the problem is more complex, and can best be understood by grouping the organisms according to their relation to all the carbohydrates studied, which has been done in Table 4.

TABLE 4.

INTESTINAL STREPTOCOCCI OF HUMAN, EQUINE, AND BOVINE ORIGIN, GROUPED ACCORDING TO THEIR FERMENTATIVE REACTIONS.

CARBOHYDRATE FERMENTED	NAME OF TYPE	PERCENTAGE OF STREPTOCOCCI FOUND IN		
		Man	Horse	Cow
None	.....	9	15	18
Dextrose alone	S. equinus	23	73	27
Lactose alone	.....	2	0	5
Dextrose and lactose	S. mitis	31	5	21
Dextrose and raffinose	.....	0	3	3
Lactose and raffinose	.....	0	0	12
Dextrose, lactose, and raffinose	S. salivarius	5	0	9
Dextrose, lactose, and mannit	S. fecalis	23	0	2
All four	.....	0	1	3

One of the most striking results of this tabulation is the confirmation it affords of the reality of the type centers established by Andrewes and Horder. The strains which fermented no carbohydrates at all may be considered as weak forms which failed to establish themselves in any of the media. Aside from this class the only large groups of organisms were those named as type centers by the English observers, Strept. equinus, Strept. mitis, and Strept. fecalis. Strept. salivarius comes next; and the other combinations of characters are exhibited by so few strains that, with one exception, they may be considered as isolated variants from the commoner types. The single exception which may prove significant is the type fermenting lactose and raffinose but neither dextrose nor mannit, which made up 12 per cent of the fecal streptococci of the cow. If further study should confirm these results this type may deserve a specific name of its own.

Reviewing the results as arranged in Table 4 it appears that equine feces contain only one common type of streptococci, Strept. equinus, which attacks dextrose but cannot ferment the other carbohydrates.

In the streptococci of human origin on the other hand there are three common types. Of our 116 human cultures, 27 fermented dextrose only, 36 dextrose and lactose, and 27 dextrose, lactose, and

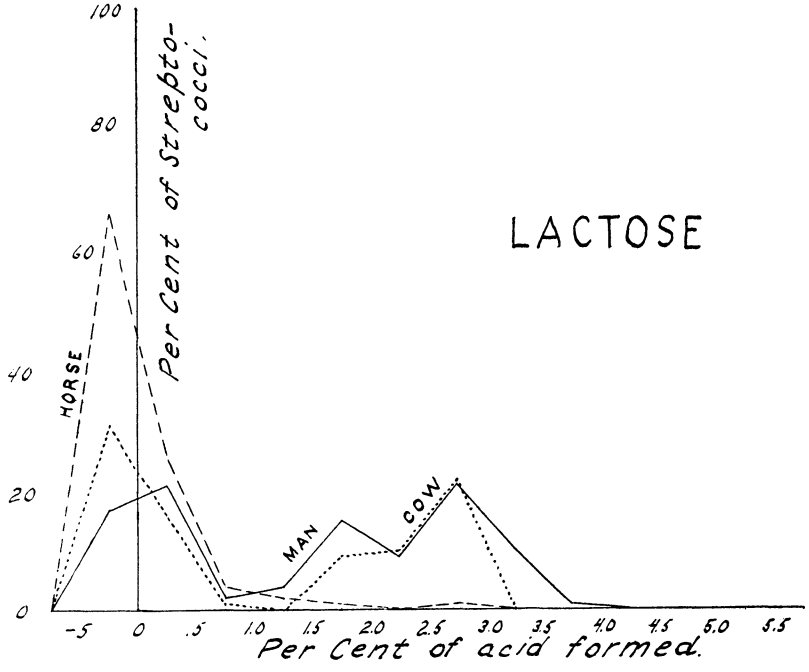

CHART 2.—Acid producing power of streptococci in lactose broth.

mannit. The first type is clearly allied to Strept. equinus; but, while these streptococci exhibit the same general qualitative relations to the sugars in the horse and man, their vigor of fermentative power, when measured quantitatively, is somewhat different. The equine strains as pointed out above produce an acidity in dextrose of about 2.0 per cent and the same is true of the bovine forms. On the other hand the human streptococci produce almost twice as much acid. There is apparently a distinct variety of Strept. equinus characteristic of the human intestine which may be recognized by its high fermentative power as measured in dextrose broth.

One interesting point about the non-lactose-fermenting streptococci of human feces, whether of the Strept. equinus type or of the group of weakened organisms which fermented neither sugar, was their association with diarrhea. We were especially anxious to see if characteristic streptococci were associated with this condition; and 8 of the 15 samples of human stools examined were more or less diarrheal in nature. Of 31 streptococci from normal stools, only 4 failed to ferment lactose; while of 85 strains from diarrheal stools

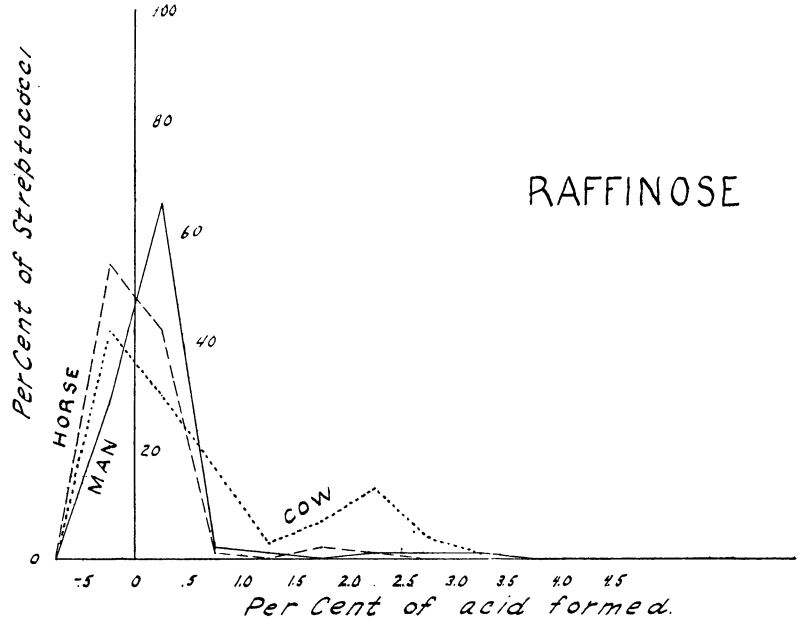

CHART 3.—Acid producing power of streptococci in raffinose broth.

36 formed no acid from that sugar. Twenty-seven of the 36 fermented dextrose vigorously and 9 were weak forms which produced no acid in any medium. The 27 strains which fermented dextrose only form an interesting group differing, as noted above, in the amount of acid produced, from the type Strept. equinus. Twenty-four of these 27 organisms came from a single individual, appearing in three different samples of diarrheal stools. Altogether 47 strains of streptococci were isolated from these three stools; 24 of them were of the Strept. equinus type and 19 of the 24 formed more than 3 per cent acid in dextrose. The other three human strains of Strept.

equinus were from the diarrheal stools of other persons; and the connection may prove to be something more than a personal idiosyncrasy.

The second type of streptococci found in human feces, the first in point of abundance, was Strept. mitis, which ferments dextrose and lactose but not raffinose and mannit. This organism was the commonest form in human feces and second in abundance in cow-dung.

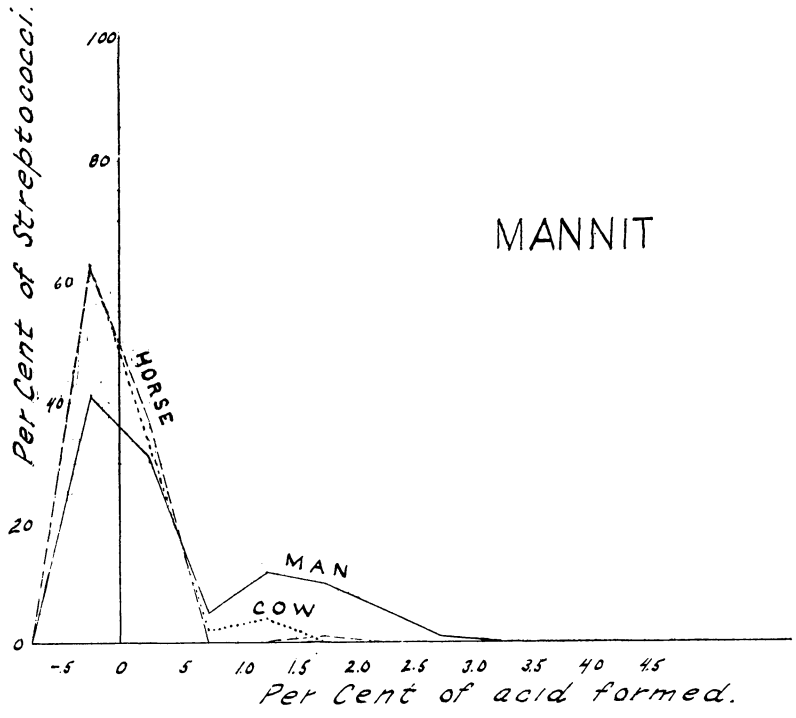

CHART 4.—Acid producing power of streptococci in mannit broth.

Finally 23 per cent of the human strains belonged to the type of Strept. fecalis, characterized by fermentation of dextrose, lactose, and mannit. This form was not found at all in the feces of the horse and but twice in that of the cow, these results corresponding closely with those obtained by Houston.

The intestinal flora of the cow appears to be more complex than either of the others. Strept. equinus and Strept. mitis are the commonest types but two other forms were also present in considerable

numbers. Nine per cent of the strains belonged to the type of Strept. salivarius, characterized by the fermentation of dextrose, lactose, and

TABLE 5.
STREPTOCOCCI ISOLATED FROM HUMAN FECES.

No.	Sample No.	Percentage of Acid Formed				No.	Sample No.	Percentage of Acid Formed			
		Dextrose	Lactose	Raffinose	Mannit			Dextrose	Lactose	Raffinose	Mannit
1	25	4.4	3.0	.1	.1	59	78	3.3	0	−.2	−.1
2	26	3.1	2.7	.4	1.7	60	78	2.9	0	+.2	−.1
3	26	3.9	3.0	.2	1.6	61	78	1.1	.2	+.2	−.2
4	26	3.6	2.8	0	.2	62	78	3.5	0	.1	0
5	27	3.4	3.0	.2	1.5	63	78	2.9	0	−.1	−.3
6	27	3.0	2.8	.2	.1	64	78	1.6	1.1	−.0	−.2
7	27	3.6	2.7	.3	.3	65	78	1.7	1.8	+.2	−.2
8	27	3.6	2.7	.3	.2	66	78	2.0	1.6	−.0	−.2
9	27	4.4	3.1	.1	.3	67	78	.1	0	−.2	−.1
10	27	3.8	2.5	.1	.9	68	78	3.2	0	−.0	−.2
11	27	3.0	3.1	.2	.4	69	78	4.4	2.7	+.2	−.1
12	27	4.1	2.9	.2	.3	70	78	.1	.1	−.0	−.2
13	27	3.8	3.0	.4	.2	71	78	0	.1	+.1	−.1
14	27	3.0	2.7	.1	.4	72	78	−.1	.1	+.1	−.1
15	27	3.3	2.8	0	1.7	73	78	2.6	0	0	1.9
16	28	3.9	3.2	0	.1	74	78	3.2	0	−.1	.8
17	28	4.3	2.8	.4	.1	75	78	3.2	0	.1	−.1
18	28	3.8	3.0	.1	1.3	76	78	3.0	0	.1	.3
19	49	4.3	2.4	.1	1.4	77	79	4.1	2.3	.3	2.6
20	49	4.0	2.8	.3	1.4	78	79	1.9	1.6	.2	1.6
21	49	4.1	2.9	.3	1.1	79	80	2.1	1.8	.2	1.6
22	49	3.9	2.7	.3	1.3	80	80	2.0	1.7	.4	1.7
23	49	4.0	3.1	0	1.2	81	80	2.0	1.7	.2	1.6
24	49	3.8	3.1	.3	1.2	82	80	2.1	1.6	.4	1.7
25	50	2.7	3.7	.9	0	83	80	2.2	.1	.3	1.5
26	50	2.3	2.8	1.4	.1	84	80	1.9	1.6	.2	1.6
27	50	2.6	3.5	1.6	0	85	80	1.9	1.7	.3	1.5
28	65	3.8	3.3	.2	.2	86	80	2.1	1.6	.3	1.7
29	65	.5	.1	.2	0	87	80	1.8	1.7	.4	1.5
30	65	4.6	3.1	.1	−.1	88	80	1.9	1.6	.2	1.5
31	65	4.2	.2	.1	.3	89	80	1.9	1.4	.4	1.8
32	65	4.5	2.3	3.2	.1	90	80	1.5	1.6	.2	1.5
33	65	.3	.2	.1	.1	91	81	2.3	2.7	.1	−.2
34	65	4.6	2.4	.2	.2	92	81	2.3	.1	.1	.1
35	65	3.6	0	.1	.3	93	81	1.7	3.0	.1	−.1
36	65	4.3	2.4	3.0	.1	94	81	−.1	2.9	0	−.1
37	65	2.7	0	.2	.1	95	81	2.5	2.8	2.6	−.3
38	65	.1	.1	.2	.2	96	81	.7	2.3	.1	−.1
39	65	2.9	0	.1	.3	97	81	2.1	1.4	−.1	−.2
40	65	4.0	.1	0		98	81	2.3	2.8	2.5	−.2
41	65	3.7	.1	.1		99	81	2.2	3.1	0	0
42	65	0	.2	0		100	81	1.6	3.1	0	.1
43	65	3.4	.1	0	0	101	88	3.4	.2	.1	.8
44	65	3.5	.1	0	−.1	102	88	3.2	1.9	0	1.1
45	65	3.5	0	−.1	0	103	88	4.5	2.1	.2	.8
46	65	3.9	0	0	0	104	88	4.8	2.4	0	−.1
47	65	3.4	0	0	0	105	88	3.6	.4	.2	.8
48	65	3.8	.1	0	0	106	88	2.9	2.2	.1	.3
49	65	3.4	.1	0	0	107	88	3.2	.5	.3	.6
50	65	.1	0	0	0	108	88	2.8	1.0	.1	.1
51	66	1.8	2.0	−.1	.1	109	89	.2	1.2	0	0
52	66	3.8	.3	.4	−.1	110	89	2.4	1.6	.2	−.1
53	66	4.9	3.3	.2	.1	111	89	2.7	−.1	.1	−.1
54	66	3.8	.3	.2	.1	112	89	0	−.2	.1	0
55	66	3.7	.1	.1	0	113	89	2.7	1.7	0	.4
56	66	5.4	3.2	.2	0	114	89	2.5	1.4	.1	.1
57	74	0	.1	−.0	−.1	115	89	2.7	.7	0	−.1
58	74	4.1	2.4	+.2	−.1	116	89	3.1	−.1	.1	−.1

raffinose. Twelve per cent of the cultures were of a new type, not apparently described hitherto, having the peculiar property of attack-

ing lactose and raffinose but not dextrose. This is highly unusual since dextrose as the simplest sugar is almost always fermented before any other carbohydrate. We have hesitated however to give a name to this type while it is characterized by only 10 cultures.

TABLE 6.

STREPTOCOCCI ISOLATED FROM HORSE-DUNG.

No.	Sample No.	Percentage of Acid Formed				No.	Sample No.	Percentage of Acid Formed			
		Dextrose	Lactose	Raffinose	Mannit			Dextrose	Lactose	Raffinose	Mannit
1	32	4.6	1.1	.8	1.9	51	46	.9	0	.1	0
2	35	1.4	1.3	.2	.3	52	46	0	.6	0	−.1
3	36	2.0	0	0	.1	53	46	1.9	.2	0	−.2
4	36	1.3	.2	0	0	54	46	1.9	−.2	0	0
5	36	1.7	.1	0	.1	55	47	2.2	2.7	.1	.1
6	36	2.1	0	.3	0	56	47	1.3	0	.1	0
7	36	.4	0	.2	0	57	47	1.9	0	0	0
8	36	2.1	0	.3	.1	58	47	2.1	−.1	0	−.1
9	36	1.9	0	0	0	59	47	1.6	−.1	0	0
10	36	.8	.6	.1	.2	60	47	1.6	.1	.2	0
11	37	2.6	0	2.8	.2	61	47	1.7	0	.1	−.1
12	37	1.1	.2	0	0	62	47	1.2	−.1	0	−.1
13	37	1.8	0	0	0	63	47	.2	−.1	−.1	0
14	37	1.0	0	.5	.3	64	47	2.4	−.1	0	−.1
15	37	2.3	0	.2	.1	65	47	2.3	.1	.2	0
16	37	2.3	0	.3	.1	66	47	2.5	0	0	1.2
17	37	1.0	0	.3	0	67	47	2.4	.1	0	0
18	37	.6	0	0	0	68	48	.2	−.4	.2	−.1
19	37	2.3	0	0	0	69	48	1.1	.3	−.1	0
20	37	2.5	0	0	.1	70	48	.1	0	.1	−.1
21	37	1.4	0	0	.1	71	48	1.5	−.2	0	−.3
22	37	1.5	0	0	0	72	48	2.2	−.3	0	−.2
23	37	1.6	0	0	.1	73	48	1.9	0	.1	−.1
24	37	1.8	1.6	0	0	74	48	2.0	0	−.1	−.2
25	38	.2	.2	0	0	75	48	1.5	.2	0	−.1
26	38	0	0	0	0	76	48	1.9	0	0	0
27	38	2.1	0	0	.3	77	48	1.8	−.1	0	0
28	38	1.4	0	.5	.1	78	48	−.1	0	0	−.2
29	38	1.5	0	.3	0	79	64	1.1	.1	0	0
30	38	1.9	0	.3	.1	80	64	1.3	0	0	.1
31	38	2.5	0	.5	0	81	82	1.0	.1	.1	.1
32	38	1.3	0	.2	.3	82	82	.8	0	0	−.1
33	38	2.0	.3	1.7	.1	83	82	1.1	0	.1	.1
34	38	2.3	0	0	0	84	82	.9	.1	.4	.1
35	38	.2	0	0	0	85	82	.8	.1	.1	0
36	38	1.9	0	0	0	86	82	.9	.1	.1	0
37	38	2.5	0	0	0	87	82	.8	0	.1	.1
38	38	2.1	0	.3	.1	88	82	1.4	.8	.2	.1
39	39	.3	.2	.3	.2	89	82	1.8	.6	.2	.1
40	39	.4	0	0	.2	90	82	1.2	−.1	.1	−.1
41	39	2.1	0	0	.1	91	82	.9	.1	0	.1
42	39	2.3	.1	0	0	92	82	1.1	0	.2	.1
43	39	1.8	.1	0	.2	93	82	.9	.1	.1	−.1
44	39	0	0	.3	.2	94	82	.8	.1	.1	0
45	39	0	0	.3	.1	95	82	.2	−.1	0	.1
46	39	0	0	.4	0	96	82	.8	.1	0	.1
47	39	1.3	0	.2	0	97	82	1.2	.1	0	.1
48	39	1.6	0	0	1	98	82	1.1	.1	−.1	0
49	39	.2	0	0	0	99	87	.9	0	−.1	−.1
50	39	2.0	.3	2.0	0	100	87	1.0	0	0	0

CONCLUSIONS.

The general result of our investigations has been to confirm and extend the conclusions of the English bacteriologists. We have found, as Andrewes and Horder concluded from their analysis of

Houston's descriptions, that the chief types of streptococci in the normal human intestine are Strept. mitis, fermenting dextrose and lactose, and Strept. fecalis, fermenting dextrose, lactose, and mannit. In addition we would call attention to the presence of a peculiarly vigorous type of Strept. equinus fermenting dextrose only.

TABLE 7.
STREPTOCOCCI ISOLATED FROM COW-DUNG.

No.	Sample No.	Percentage of Acid Formed				No.	Sample No.	Percentage of Acid Formed			
		Dextrose	Lactose	Raffinose	Mannit			Dextrose	Lactose	Raffinose	Mannit
1	41	2.1	2.0	0	0	44	69	3.0	.2	0	0
2	41	.6	0	0	0	45	69	0	.1	.1	.1
3	43	2.7	2.5	.1	0	46	69	.1	0	0	.1
4	44	3.4	3.4	1.2	.1	47	69	0	.2	0	0
5	44	2.9	2.2	0	0	48	69	2.0	2.0	0	0
6	44	2.1	2.1	0	.1	49	69	2.5	.2	.1	0
7	44	2.3	2.1	0	0	50	69	.2	.1	0	0
8	44	.2	3.0	2.6	0	51	69	2.5	.1	−.1	.1
9	53	2.3	2.4	2.0	0	52	69	0	2.0	0	0
10	53	0	3.1	2.0	0	53	69	2.5	1.7	−.1	0
11	53	4.1	2.9	0	.8	54	69	.1	.3	.1	0
12	54	2.2	2.6	1.7	0	55	69	.1	.1	0	0
13	54	2.1	2.6	2.0	.1	56	69	2.2	.1	−.1	0
14	54	−.1	−.2	−.1	−.1	57	70	2.5	−.1	.1	.1
15	54	−.1	2.9	−.1	0	58	71	2.3	0	.2	.1
16	54	0	3.1	2.0	−.1	59	71	2.5	0	0	−.1
17	54	−.2	3.0	2.3	1	60	71	2.4	−.1	.1	0
18	54	−.1	2.8	2.3	0	61	71	.1	−.1	.2	−.1
19	54	−.1	2.9	2.4	.1	62	71	2.5	−.1	.1	0
20	54	3.1	2.7	−.1	.1	63	71	2.8	0	.1	0
21	54	−.2	2.8	−.1	0	64	71	2.0	0	.1	−.1
22	54	3.3	3.0	.1	−.2	65	72	3.0	1.6	.2	.1
23	56	2.1	−.1	2.1	0	66	72	.3	−.1	0	−.1
24	56	3.7	2.6	−.1	1.4	67	72	3.3	−.1	0	.2
25	56	2.4	2.5	2.3	−.1	68	72	2.6	2.0	.1	−.1
26	56	0	2.9	2.3	.2	69	72	2.5	−.1	.1	.1
27	56	−.1	3.1	2.4	.1	70	72	2.4	.1	2.6	0
28	56	−.1	2.5	2.3	.1	71	72	2.2	−.2	.1	0
29	56	4.2	3.1	2.0	1.3	72	72	0	0	.0	0
30	56	0	2.9	2.3	0	73	72	2.2	0	.1	.2
31	56	3.5	2.9	2.5	−.1	74	72	1.9	−.1	.1	.1
32	59	3.5	2.9	0	.1	75	75	4.1	2.7	.2	0
33	61	3.5	2.9	0	0	76	77	3.7	.6	.1	−.1
34	61	4.2	3.1	0	.1	77	86	1.0	0	.1	.1
35	61	3.9	3.2	3.5	0	78	91	−.2	.1	.1	.1
36	61	3.9	3.5	0	0	79	91	.1	0	.2	.2
37	67	2.0	.2	0	0	80	92	2.3	1.6	1.4	1.3
38	67	1.1	.2	0	0	81	92	2.2	1.9	1.5	.9
39	68	.4	0	.1	0	82	92	.1	0	.1	.2
40	68	2.8	.2	−.1	0	83	96	.2	0	.2	0
41	68	2.6	.1	0	0	84	96	1.7	1.2	2.3	.1
42	68	0	2.3	−.1	0	85	98	2.8	2.6	3.0	0
43	69	2.0	0	0	.1	86	97	.3	0	0	−.1

We have found, as Andrewes and Horder did, that the characteristic streptococcus of the horse is the non-lactose-fermenting Strept. equinus; and this appears to be the only form typically present. Seventy-three per cent of all our equine strains belonged clearly to this type.

In the feces of the cow, on the other hand, streptococci which fail

to ferment lactose are relatively less common, as Houston showed. Strept. equinus is present; but so are Strept. mitis and Strept. salivarius (fermenting dextrose, lactose, and raffinose); and we have found in small numbers a peculiar new type fermenting lactose and raffinose but not dextrose. Strept. fecalis, as in the horse, is absent.

From the standpoint of the water bacteriologist several conclusions may be drawn. In the first place, it appears that pollution with road washings may be distinguished from wastes of other sorts by a study of the streptococci present. Since most of the pollution in street washings comes from horse-dung, and since lactose-fermenting streptococci are comparatively rare in such deposits, a test for these organisms should have distinct value. Such a test might easily be made by inoculating tubes of lactose broth, incubating for several days, and then plating on lactose agar.

The distinction between human and bovine pollution is also promising. There are three points of difference which seem to deserve investigation. First the presence of streptococci forming over 3.5 per cent of acid in dextrose broth would seem in general to be characteristic of human stools. Second, raffinose-fermenting forms (Strept. salivarius) appear to be more abundant in bovine than in human feces. Third, and of most importance, mannit-fermenting streptococci (Strept. fecalis), which make up about one-quarter of the human streptococci, are very rare in the feces of the horse and cow. In this respect Houston's results and our own are in complete agreement; and the use of mannit broth as a differential test for streptococci of human origin would seem sufficiently promising to warrant further study.

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